Remarks

Objection

The Examiner has objected to claim 4 in that it contains the phrase "stranced RNA". As suggested by the Examiner, this is a typographical error. The claim has been corrected to recite "stranded RNA". Applicant thanks the Examiner for pointing out this typographical error.

Rejections under 35 U.S.C. § 112

Claims 1-35 and 67-101 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

The Examiner has pointed out that the phrase "the two pools" in claims 1 and 67 has no antecedent basis. Claim 1 has been amended to recite "A method of sequencing a nucleic acid molecule comprising steps of: providing two separate, adjacent pools of a medium..." rather than "solutions of a medium" as in the original claim, thereby providing antecedent basis for the phrase "the two pools". Claim 67 has been similarly amended to recite "A method of sequencing a nucleic acid molecule comprising steps of: providing two separate, adjacent pools of a medium..." rather than "solutions of a medium" as in the original claim. Support for these amendments is found at p. 43, lines 2-3 (paragraph 117), which recites, "Nanopore sequencing involves the use of two separate pools of a medium and an interface between the pools.", at p. 49, lines 19-21 (paragraph 132), which recites "Nanopore sequencing is capable of sequencing double stranded or single stranded nucleic acids, by (1) providing two separate, adjacent pools of a medium and an interface (e.g., a lipid bilayer) between the two pools...", and elsewhere in the specification. Withdrawal of the rejection in view of this amendment is respectfully requested.

The Examiner has asserted that claims 12-13, 16-17, 25-26, 82-83, 86-87, and 95-96 are vague and indefinite on the ground that "it is unclear what is the definition of the phrase 'ionic flow blockage' in the specification". The phrase is used in the specification at p. 50, lines 2-5 (paragraph 132), which describes a method of nanopore sequencing, which includes a step of "(3) taking measurements (e.g., ionic flow measurements, including measuring duration or amplitude of ionic flow blockage) as each of the nucleotide monomers of the nucleic acid nucleic

acid passes through the channel..." The specification explains at p. 51, line 22 – p. 52, line 4 (paragraph 138) that in one embodiment, "nanopore sequencing involves measurements of ionic current modulation as the monomers (e.g., nucleotides) of a linear nucleic acid (e.g., nucleic acid molecule) pass through or across a channel...During nucleic acid passage through or across the channel, ionic currents are reduced in a manner that reflects...the identities of the monomers." At p. 56, lines 11-13 (paragraph 155), the specification further explains that, "The nucleotide bases of a DNA molecule, for example, passing through or over the opening of a channel protein, disrupt the flow of ions through the pore..." It is thus clear that the phrase "ionic flow blockage" as used in the specification and claims refers to a disruption or obstruction in the movement of ions through a channel, such that the flow of ions through the channel is reduced or eliminated. Such blockages can occur, for example, as a strand of nucleic acid or other polymer passes through the channel. The detection of such a decrease in ion flow is one means by which, for example, the identity of a nucleotide passing through the channel or the concentration of a polymer in solution can be determined.

The specification also uses related terminology that further confirms the above interpretation of the term "ionic flow blockage". As is well known in the art, a flow of ions establishes a current, and a channel or device through which ions flow is said to have a conductance, the magnitude of which is a measure of the ability of the channel or device to carry a current. The specification makes reference to "conductance blockade" and "current blockage events" at p. 55, line 21 - p. 56, line 3 (paragraph 152), which recites "Concentration of polymers can be rapidly and accurately assessed by using relatively low resolution recording conditions and analyzing the number of conductance blockade events in a given unit of time. This relationship should be linear and proportional (the greater the concentration of polymers, the more frequent the current blockage events)..." It is clear from the specification that "ionic flow blockage", "conductance blockade", and "current blockage" all refer to the same phenomenon, i.e., a disruption or obstruction in the movement of ions through a channel per unit time.

The term "ionic flow blockage" would readily be understood by one of ordinary skill in the art to mean a disruption or obstruction in the movement of ions through a channel, such that the flow of ions through the channel is reduced or eliminated. Indeed Baldarelli, which is incorporated by reference into the specification, uses the term extensively in his description of a method for sequencing and also in the claims. His use of the term is substantially identical to the

use of the term in the instant specification. For example, Applicant notes the use of the term in a large number of Baldarelli's claims (e.g., claims 6, 7, 10, 11) and his numerous references to blockages and blockades in his Brief Description of the Drawings, all referring to events that disrupt or obstruct the flow of ions through a channel.

The above interpretation is also consistent with the ordinary meaning of the words "ionic" (of, containing, or involving ions), "flow" (a continuous movement or outpouring), and "blockage" (the act of obstructing) (The American Heritage Dictionary, Second College Edition, Houghton Mifflin Company, Boston, 1985).

For all of these reasons, withdrawal of the rejection is respectfully requested.

Rejections under 35 U.S.C. § 103

Claims 1-34 and 67-100 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Baldarelli et al. (6,015,714), hereinafter "Baldarelli" in view of Sampson et al. (6,054,274), hereinafter "Sampson". Baldarelli discloses a method of nucleic acid sequencing that involves passing a nucleic acid strand through a pore or channel. Sampson teaches a method of amplifying the signal of a target nucleic acid analyte by using rolling circle replication to attach detectable tags to nucleic acids.

As set forth in MPEP §706.02(j), Contents of a 35 U.S.C. §103 Rejection, "To establish a *prima facie* case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." As discussed in further detail below, neither Sampson nor Baldarelli teaches or suggests that modified nucleotides could be used to reduce secondary structure. Even if some of the modified nucleotides taught by Sampson could be used to reduce secondary structure in some contexts, Sampson provides no guidance as to which modified nucleotides could be used or how they might be used to achieve this result. There is no indication that a nucleic acid molecule incorporating one or more of the modified nucleotides taught by Sampson would have reduced secondary structure as required by the instant claims.

As pointed out by the Examiner, Sampson teaches the existence of modified nucleotides.

However, modified nucleotides are merely mentioned in the definition of "oligonucleotide", and Sampson does not discuss their properties or suggest that they might be used to reduce secondary structure of a nucleic acid. Thus Sampson does not teach that these modified nucleotides are "modified nucleotides that reduce secondary structure in the nucleic acid molecule", as required by independent claims 1 and 67. Sampson merely teaches that oligonucleotides can include modified nucleotides. Furthermore, the fact that Sampson mentions modified nucleotides only in the context of the definition of "oligonucleotide", while omitting mention of modified nucleotides in the definition of "dNTP" (col. 5, lines 19-21), would suggest to the skilled artisan that modified nucleotides might possibly be relevant to the particular context in which Sampson employs oligonucleotides, but would not suggest their use more generally, e.g., in other contexts such as in nucleic acids to be sequenced.

As pointed out by the Examiner, Sampson states that inclusion of "nonstandard" A-G and G-A base pairs "within the sequence context of the stem structures results in the complementary circular DNA template not having any predicted secondary structure" (col. 9, lines 15-19). However, the A and G nucleotides that Sampson refers to are not modified nucleotides, as required by the instant claims. Instead, they are simply the A and G nucleotides normally found in DNA and RNA. Sampson is not using the term "nonstandard" to refer to the nucleotides themselves (e.g., to indicate that they are nonstandard nucleotides) but only to the fact that an A-G or G-A base pair is nonstandard because A does not normally pair with G.

Sampson's teachings with respect to A-G and G-A base pairs do not suggest the use of modified nucleotides for the purpose of reducing the secondary structure of a nucleic acid. The presence of A-G and G-A base pairs in the stem structures would result in T-C and C-T at corresponding positions in the complementary circular template. Such base combinations are not thermodynamically stable and would not contribute to secondary structure formation. However, T and C are not nonstandard nucleotides, and Sampson merely teaches what is well known in the art, namely that T and C do not form thermodynamically stable base pairs. Thus one of ordinary skill in the art reading Sampson would find no motivation to utilize modified nucleotides for the purpose of reducing secondary structure.

In addition, Sampson indicates that the effects of the presence of A-G and G-A base pairs on secondary structure depends on the sequence context in which they are found. Sampson states that "the stem structures are composed of standard G-C base pairs and two non-standard

A-G and G-A base pairs that are known to be thermodynamically stable when presented in the given context..." (col. 8, lines 62-65)." Thus one of ordinary skill in the art reading Sampson would learn that in some contexts A-G and G-A base pairs are stable while in other contexts they are not. Sampson does not describe which sequence contexts result in stability and which do not, and it is unclear whether such sequence contexts would exist in a nucleic acid to be sequenced. In addition, it is unclear how one would engineer a nucleic acid to be sequenced so as to position A-G or G-A base pairs in the correct sequence context to reduce the secondary structure of the nucleic acid since doing so clearly requires knowledge of the sequence of the nucleic acid. If that knowledge was available, then presumably the skilled artisan would not be sequencing the nucleic acid in the first place. Therefore, the skilled artisan reading Sampson would not know how to utilize A-G and/or G-A base pairs, or any other base pairs, to achieve the desired reduction in secondary structure.

Even if Sampson did suggest the use of modified nucleotides for the purpose of reducing secondary structure, he provides absolutely no guidance as to how such a result might be achieved. Sampson merely mentions a variety of modified nucleotides that may have modified bases or sugar groups. He provides no indication which, if any, of these modified nucleotides would actually be usable to reduce secondary structure or how to use such nucleotides to actually achieve the desired result. As taught in the instant specification, suitable modified nucleotides for creating an unstructured nucleic acid (UNA), "have a reduced ability (or no ability) to form base pairs with a complement which is also incorporated into the UNA (but) must be capable of forming a base pair with a different yet still complementary nucleotide" p. 28, lines 6-10 (paragraph 78). Sampson does not indicate which, if any, of the modified nucleotides he mentions would be able to base pair either with each other or with an unmodified nucleotide. He does not teach or suggest that any of the modified nucleotides would have reduced base pairing ability relative to standard nucleotides. He does not teach which, if any, of the modified nucleotides would have reduced base pairing ability with respect to certain complements while still retaining ability to form a base pair with different complementary nucleotides, which the instant specification teaches should be the case in order to achieve reduced secondary structure in a nucleic acid to be sequenced.

Even if some of the modified nucleotides taught by Sampson might have reduced ability to base pair with one or more other standard or nonstandard nucleotides, Sampson does not teach

how to incorporate such nucleotides into a nucleic acid so as to actually achieve a reduction in secondary structure, how many should be used, or which combinations of modified and/or unmodified would be effective. For example, the instant specification teaches that a nucleic acid to be sequenced, which contains a modified nucleotide, should also include a complementary nucleotide with which the modified nucleotide has reduced base pairing ability. The instant specification teaches various combinations of modified nucleotides that are effective for reducing secondary structure. Such teachings are entirely lacking in Sampson.

In summary, Sampson does not teach that any of the modified nucleotides he mentions would reduce secondary structure of a nucleic acid, as required by the instant claims, and there is no teaching or suggestion in either Sampson or Baldarelli that modified nucleotides of any kind could be used to reduce secondary structure of a nucleic acid. Therefore, one of ordinary skill in the art would have had no motivation to combine the use of modified nucleotides, as mentioned by Sampson, with the teachings of Baldarelli that it is preferable to avoid secondary structure in the nucleic acid to be sequenced. Furthermore, even if Sampson did suggest the use of modified nucleotides for generating nucleic acids with reduced secondary structure, his teachings are entirely lacking in enablement since he provides no guidance as to which modified nucleotides to select, how many to use, what sort of base pairing they might undergo, or the strength of such base pairs relative to the strength of base pairs normally found in DNA or RNA. In the absence of such guidance the skilled artisan would have had no reasonable expectation of success if he or she attempted to use these nucleotides to reduce secondary structure of a nucleic acid to be sequenced. Thus Applicant submits that none of the three criteria necessary for a *prima facie* case of obviousness have been met.

Applicant further points out that the Examiner has not explained the basis for the rejection of claims 31-33 and 74-77 in view of Baldarelli and Sampson. Claims 31 and 74 require that, "the nucleic acid molecule contains modified adenosine and modified thymine which are not able to form base pairs, wherein the modified adenosine is capable of forming a base pair with unmodified thymine, and wherein the modified thymine is capable of forming a base pair with unmodified adenosine". Claims 32 and 75 require that, "the nucleic acid molecule contains modified guanosine and modified cytosine which are not able to form base pairs, wherein the modified guanosine is capable of forming a base pair with unmodified cytosine, and wherein the modified cytosine is capable of forming a base pair with unmodified guanosine".

Claims 33 and 76 require that, "the nucleic acid molecule contains 2-aminoadenosine, 2-thiothymidine, inosine, and pyrrolopyrimidine". Claims 34 and 77 require that the nucleic acid molecule contains 2-aminoadenosine, and 2-thiothymidine". These claim limitations are not taught or suggested by either Sampson or Baldarelli.

In conclusion, the combination of Baldarelli and Sampson is insufficient to establish any of the three criteria required to make out a *prima facie* case of obviousness. In the instant case, there is no motivation to combine Baldarelli and Sampson since neither of them suggests that modified nucleotides might be used to reduce secondary structure of a nucleic acid. There is no reasonable expectation that using the modified nucleotides of Sampson would result in a nucleic acid with reduced secondary structure. Neither Baldarelli nor Sampson either alone or in combination teaches or suggests that any of the particular modified nucleotides they disclose meet the limitation in claims 1 and 67 requiring the use of modified nucleotides that actually do reduce secondary structure in the nucleic acid molecule. Neither Baldarelli nor Sampson teaches or suggests the further limitations present in claims 31-33 and 74-77. Given that not a single criterion for a *prima facie* case of obviousness is met, Applicant respectfully requests withdrawal of the rejection.

Claims 35 and 101 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Baldarelli in view of Sampson as applied to claims 1-34 and 67-100, and further in view of Thorp (5,871,918), hereinafter "Thorp". The Examiner points out that Thorp discloses a method of detecting a nucleic acid by use of tunneling and therefore asserts that the use of tunneling to detect a nucleic acid as taught in the instant claims would be obvious. As explained above, Baldarelli and Sampson do not render claims 1-34 or 67-100 obvious. In particular, Sampson does not teach or suggest modified nucleotides that could be used for purposes of reducing secondary structure as required by claims 35 and 101 through their dependency on claims 1 and 67. The Examiner has not suggested that Thorp adds anything to the combination of Baldarelli and Sampson with respect to the use of modified nucleotides to reduce secondary structure. Therefore, the combination of Baldarelli, Sampson, and Thorp does not render claim 35 or claim 101 obvious. Withdrawal of the rejection is respectfully requested.

In conclusion, in view of the amendments and remarks presented herein, the application and pending claims comply with the requirements of 35 U.S.C. §101 and §112. Applicant therefore respectfully submits that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

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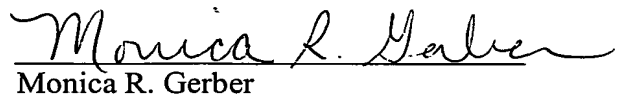
Respectfully submitted,



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